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GRANT NUMBER DAMD17-95-1-5031

TITLE: The Development of an Effective Vaccine, Post Exposure Prophylaxis and for Prevention and Control of AIDS

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REPORT DATE: May 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. AGENCY USE ONLY *(Leave blank)* | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | May 1998 | Annual (1 May 97 - 30 Apr 98) |

**4. TITLE AND SUBTITLE**
The Development of an Effective Vaccine, Post Exposure Prophylaxis and for Prevention and Control of Aids

**5. FUNDING NUMBERS**
DAMD17-95-1-5031

**6. AUTHOR(S)**
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**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

**10. SPONSORING/MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for public release; distribution unlimited

**12b. DISTRIBUTION CODE**

**13. ABSTRACT** *(Maximum 200 words)*

Studies undertaken in Year 3 of Grant DAMD 17-95-1-5031 focused on identification of peptide mimetics capable of generating broadly reactive neutralize antibodies versus globally diverse primary isolates of HIV-1. More than one hundred synthetic peptides were screened for immunogenicity and neutralization of a B clade primary isolate. From this initial screen, four peptides designed to mimic the neutralizing antibody profit of monoclonal antibody B4 were identified in preclinical immunogenicity studies and found to be capable of stimulating effective neutralizing antibody. Two of the four peptides were further evaluated and were found to be effective in stimulating antibodies which neutralize primary isolates of HIV-1 from clades A-E, SHIV 89.6 and 89.6P, HIV-1 chimp isolate4Dh-12, and the laboratory strain SIVmac251. Concurrently development studies continued on monoclonal antibody B4 as a post-exposure prophylactic, and studies with B4 in chimpanzees demonstrated it was effective in preventing establishment of DH-12 infection of chimps when added 1 hour post virus challenge.

| 14. SUBJECT TERMS | 15. NUMBER OF PAGES |
|---|---|
| Prophylaxis, Immunotherapy, Post Exposure, Effective Vaccine | 23 |
| | 16. PRICE CODE |

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | Unclassified | Unlimited |

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Wayne C Reff 5/22/98

PI - Signature Date

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## 5. **Introduction**

5.1. Background (Adapted from Proposal and from 1996-97 Annual Report)

The World Health Organization (WHO) estimates that by the year 2000, 40 million persons worldwide will be infected with human immunodeficiency virus (HIV), the virus which causes AIDS. Despite global investments in HIV prevention and control, HIV infection rates are expanding in several regions of the world. From both a civilian and military perspective, the development of safe and effective HIV vaccines and therapies has become an international public health priority.

The scientific obstacles to HIV/AIDS prevention and control efforts are many and include: multiple genetic subtypes of HIV circulating worldwide; multiple routes of HIV transmission; efficient transmission of HIV by cell-free and cell-associated virus; lack of complete understanding of which immune responses are required for protective immunity; and the development of virus resistance to licensed antiviral therapies (Myers. et. al., 1992; Levy; 1992; Koff, 1988; Koff 1994). In addition, the qualities of the HIV envelope glycoproteins which have made generation of effective humoral immunity versus primary isolates quite challenging, coupled with the capacity of HIV to integrate into host cells leading to increased diversity upon subsequent rounds of activation, provide additional obstacles to successful HIV prevention and control.

United Biomedical, Inc. (UBI) has developed a comprehensive vaccine and immunotherapy program focused on a multicomponent strategy aimed at eliciting complementary elements of the host immune system i.e. humoral, cellular and mucosal immune responses which are necessary for effective prophylaxis and treatment of HIV/AIDS. Research supported by Grant# DAMD17-95-1-5031 provides support for several aspects of this program.

During year 2 of Grant# 17-95-1-5031 (May 1, 1996-April 30, 1997), research focused on:

1. Completion of Phase 1 HIV Immunotherapy studies using prototype lipopeptide immunogen.

2. Completion of Phase 1 microparticle studies evaluating combinations of oral + parenteral administration of immunogens for induction of systemic and mucosal immunity.

3. Evaluation of methods to optimize CTL responses utilizing DNA plasmids and synthetic peptide immunogens.

4. Screening of immunogens for induction of neutralizing antibodies against primary HIV isolates, utilizing monoclonal antibody 95-29-5 (also termed B4) as the benchmark.

5. Continuation of preclinical studies aimed at improving duration of immunity and stimulation of mucosal immune responses, utilizing microparticles and mucosal adjuvants.

5.2. Summary of major observations from year 2 (adapted from 1996-97 annual report)

• Synthetic lipopeptides as immunotherapeutics are well tolerated in HIV-infected subjects

• Oral immunization of microparticles containing HIV-MN branched peptide, when preceded by parenteral priming of HIV-MN branched peptide is safe in healthy HIV-negative adult volunteers. Immunogenicity studies of parenteral priming +oral microparticles boosting does not provide superior systemic immune responses compared with oral immunization alone; Antigen specific immune responses as determined by ELISPOT for IgA on heparinized peripheral blood specimens and mucosal binding assays for IgA on parotid saliva samples demonstrated the immunogenicity of the parenteral priming +oral microparticle regimen for generating HIV-specific IgA responses by synthetic peptide vaccination.

• DNA immunization provides a robust strategy for stimulating HIV-specific CTL responses, which can be augmented by co-administration of plasmids expressing IL-12; Immunization regimens consisting of DNA priming for generating cellular immunity plus peptide boosters for stimulating high levels of humoral immunity warrants further study based on our observations in murine and primate models.

• Monoclonal antibody B4 is effective at neutralizing primary isolates of HIV-1 in all clades (A-F) thus far tested; B4 is effective at neutralizing HIV-1 when added pre or post infection, and SCID-Hu mice studies demonstrated protective efficacy in a post-infection prophylaxis model; Preliminary findings with a synthetic immunogen derived from binding studies with Mab B4 indicate that it should be feasible to develop immunogens capable of neutralizing primary isolates of HIV-1.

• Preliminary studies aimed at generating mucosal immune responses in the female genital tract by modifying routes of immunization, coupled with mucosal adjuvants, demonstrated that systemic priming followed by intravaginal boosting is capable of stimulating both HIV-specific IgG and IgA in uterine secretions along with IgG in serum.

Studies being undertaken in this grant are focused on issues central to the development of safe and effective HIV vaccines and immunotherapies, including induction of the requisite cellular, humoral and mucosal immune responses. In the absence of well-

defined correlates of protective immunity for HIV infection and AIDS, we have taken the approach to try to maximize the following immune responses:

A. Humoral immune responses capable of neutralizing primary isolates of HIV from divergent internationally circulating clades of HIV.

B. Cellular immune responses capable of conferring long-term memory, and also capable of functionally targeting and killing HIV-infected cells.

C. Mucosal immune responses capable of thwarting infection at local sites of HIV entry, with a specific emphasis on inhibiting sexual transmission of HIV.

Studies in year 2 (May 1996-April 1997) of this grant have addressed the following common strategies:

o Combinations of systemic priming plus mucosal boosting may be necessary to induce the targeted and compartmentalized immunes responses discussed above.

o Combinations of vaccine strategies (e.g. DNA priming + subunit boosting) may be necessary to stimulate high levels of both CTL and neutralizing antibodies.

o Combinations of virus-specific and virus-receptor specific antigens may be necessary to elicit high levels of HIV-specific CTL and neutralizing antibodies.

Based on studies conducted in the first two years of this grant, the following goals were studies were proposed for year 3 of the grant (May 1, 1997-April 30, 1998) :

1. Further development of synthetic immunogens capable of neutralizing primary isolates of divergent clades of HIV-1.

2. Combination studies of DNA immunogens + subunit boosts (peptides and lipo-peptides) for maximizing CTL and humoral immunity.

3. Combination studies of systemic priming +mucosal boosting utilizing immunogens evaluated in Aim #2 above to generate significantly greater levels of mucosal immunity to supplement cellular and humoral immune responses.

## 6.0 **BODY**

Based on the aims described above, we made a programmatic decision to focus our efforts on specific aim #1: **Further development of synthetic immunogens capable of neutralizing primary isolates of divergent clades of HIV-1** during year 3 (May 97 to April 98), since combination studies designed for optimizing cellular/humoral immunity (Aim #2 above) or mucosal humoral immunity (Aim #3 above), would be contingent on the successful demonstration that such immunogens would be effective in neutralizing diverse primary isolates.

Data presented in last year's progress report described the development of monoclonal antibody B4, with the following properties:

- o neutralizing primary isolates of HIV-1 in all clades (A-F) thus far tested
- o neutralizing HIV-1 when added pre or post infection
- o protective efficacy versus HIV in SCID-Hu mice in a post-infection prophylaxis model.

Based on these promising observations, studies in Year 3 focused on the development of synthetic immunogens capable of stimulating anti-HIV humoral immunity which mimics the efficacy of B4 in neutralizing diverse primary isolates, and extending the observations on B4 to evaluation of HIV prevention and post-exposure prophylaxis in an HIV-chimpanzee model of infection with the HIV primary isolate DH-12.

### 6.1 Development of synthetic immunogens capable of neutralizing primary isolates of divergent clades of HIV-1

MATERIALS AND METHODS

Animals. Duncan Hartley guinea pigs were used for screening synthetic peptide immunogens.

Synthetic peptides. Peptides were synthesized by the Merrifield solid-phase synthesis technique on Applied Biosystems automated peptide synthesizers (Models 430, 431 and 433A) using Fmoc chemistry. After complete assembly of the desired peptide, the resin was treated according to standard procedure using triflouroacetic acid to cleave the peptide from the resin and deblock the protecting groups on the amino acid side chains. The cleaved, extracted and washed peptides were purified by HPLC and characterized by mass spectrometry and reverse phase HPLC.

Peptide Immunogen Design. Based on our observations with monoclonal B4, which was derived by immunization of mice with HPB-ALL cells, a human T cell lymphocytic leukemia cell line (See Annual Report, Year 2, May 96-April97), peptides were designed to mimic the following host cell antigens: human CD4, CCR1, CCR2b, CCR3, CCR5 and CXCR4. Peptide immunogens were designed with a spectrum of peptide technologies including precise epitope mapping, cyclic constraints, and incorporation of

promiscuous T-helper epitopes and immunomodulatory domains for enhanced immunogenicity.

Standard Immunization Screening Protocol. Individual peptide immunogens or equi-molar ratios of peptide immunogen mixtures, were injected i.m. at 100μg in 0.5ml either in CFA/IFA or alum adjuvant. For initial screening, a 0,3, 6 week protocol or 0,2, 4 week protocol was used. Animals were bled on week 8, and evaluated by anti-peptide ELISA and viral neutralization.

Anti-peptide enzyme-linked immunosorbent assays. Anti-peptide activities were determined by ELISA using 96-well flat-bottom microtiter plates, which were coated with the corresponding target antigenic site as immunosorbent. Aliquots (100μl) of a target antigenic peptide solution at a concentration of 5μg/ml were incubated for 1 hour at $37^0$ C. The plates were blocked by another incubation at $37^0$ C for 1 hour with a 3% gelatin/PBS solution. The blocked plates were then dried and used for the assay. Aliquots (100μl) of the test immune sera, starting with a 1:100 dilution in a sample dilution buffer and ten-fold serial dilutions thereafter, were added to the peptide coated plates. The plates were incubated for 1 hour at $37^0$ C. The plates were then washed 6x with 0.05% PBS/Tween buffer. 100μl of horseradish peroxidase labeled goat-anti-species specific antibody was added at appropriate dilutions in conjugate dilution buffer (Phosphate buffer containing 0.5M NaCl, and normal goat serum). The plates were incubated for 1 hour at $37^0$ C before being washed as above. 100μl of o-phenylenediamine substrate solution was then added. The color was allowed to develop for 5-15 minutes before the enzymatic color reaction was stopped. Absorbance of the contents of each well at $A_{492nm}$ was read in a plate reader. ELISA titers were calculated based on linear regression analysis of the absorbances, with cutoff $A_{492nm}$ set at 0.5. This cutoff value was rigorous, as the values for diluted normal guinea pig control samples run with each assay were less than 0.15.

Indirect immunofluorescence assays. $0.5 \times 10^6$ HPB-ALL or MT-2 cells per well were washed twice in PBS containing 1% BSA prior to their incubation with the designated immune sera or monoclonal antibodies, at an optimal concentration as determined for each experiment, for 45 minutes at room temperature. After incubation of cells with the first staining antibody, the cells were washed for an additional two times in the same washing buffer and were incubated with a secondary FITC conjugated goat anti-mouse IgG or FITC conjugated goat anti-guinea pig IgG for an additional 45 minutes at room temperature. The stained cells were washed again in the same washing buffer and the cells processed for fluorescence analysis by cytofluorograph and/or immunofluorescence microscopy for determination of percentage of stained cells, and intensity of staining. For competitive inhibition assays aimed at inhibiting the binding of B4 to HPB-ALL or MT-2 cells, cells were first incubated with the sear from peptide immunized guinea pigs, and washed twice in the same washing buffer before the addition of biotinylated monoclonal antibody B4. Staining was completed by subsequent incubation with

appropriately diluted FITC-avidin followed by additional three washes prior to analysis by cytofluorograph or high resolution fluorescence microscope.

MT-2 microplaque neutralizing antibody assay. In collaboration with Dr. Carl Hanson of the Viral and Rickettsial Disease Laboratory of Berkeley, California, (Hanson, CV et. al. 1990) HIV neutralization assays were conducted in MT-2 microplaque format. Briefly, heat-inactivated sera are serially diluted in diluent consisting of 50% high glucose DMEM with 15% FBS, antibiotics, 2% glutamine and bicarbonate buffer, and 50% pooled, defibrinated normal human plasma. The large proportion of normal human plasma in the diluent is designed to overwhelm any non-specific stimulatory or inhibitory effects of the test sample. Serum dilutions are transferred in quadruplicate to flat-bottomed 96 well plates and mixed with an equal volume of virus, which has been diluted to 15-20 PFU per well in the same diluent. The serum and virus are incubated for 1-18 hours at $37^0$ C in a humidified $CO_2$ atmosphere. Ninety thousand MT-2 cells per well are then added and incubated with the serum/virus mixture for one hour. Warmed growth media containing 1% Seaplaque agarose (FMC Corp.) is added, quickly mixed, and the plates are centrifuged at 500 x g for 20 minutes at $20^0$ C. Cell monolayers form on the bottom of the well due to centrifugal force prior to gelling of the agarose. Plates are incubated 6 days, stained with propidium iodide, and the plaques are counted 24 hours later. The antibody dilution resulting in 50% or 90% plaque reduction is determined by computerized interpolation.

RESULTS

Peptides aimed to induce HIV neutralizing antibody similar to that demonstrated by monoclonal antibody B4 were designed and screened by anti-peptide ELISA. In the initial screening of peptide immunogens, linear peptides consisting of T-helper epitopes linked to putative antigenic sites (B-cell epitopes) on host cellular antigens representing the HIV receptor/co-receptor (see Materials and Methods), generated significant levels of anti-peptide antibody, and anti-host cell antigen antibody (by indirect immunofluorescence), but no neutralizing antibody against primary isolates of HIV. Table 1 shows a representative experiment for six peptides, with more than one hundred peptides screened by this experimental format. Similarly, cyclization of peptide immunogens did not *a priori* lead to immunogens with the capability of neutralizing HIV primary isolates (Table 2).

In an effort to mimic the efficacy of B4, competitive inhibition assays were undertaken with sera from guinea pigs immunized with peptide immunogens, with the goal of blocking B4 binding and thus gaining insight into potential immunogens with B4 "mimotope" functionality. Based on these studies, four peptide mimetics were generated and shown to elicit neutralizing antibodies versus HIV primary isolates. (Table 3). To our knowledge, this was the first demonstration of a peptide immunogen capable of stimulating anti-HIV neutralizing antibodies, and thus a patent application is in the process of being drafted for submission. (NOTE: Due to the early stage of patent

development, sequences identifying the peptide immunogens effective in neutralizing HIV primary isolates are not included in this annual report).

From the four peptide mimetics with anti-HIV activity, two was selected for further development, and shown to be capable of neutralizing multiple clades of HIV-1, in a similar profile to monoclonal antibody B4 (Table 4). Moreover, studies with SHIV isolates SHIV89.6 and the pathogenic SHIV89.6P demonstrated that sera from guinea pigs immunized with this peptide mimetic could also neutralize these isolates (Table5), albeit at titers significantly less than those observed with B4.

In summary, prototype peptide immunogens have been identified which when immunized in guinea pigs elicits antibodies capable of neutralizing multiple clades of primary isolates of HIV-1. Future development studies, aimed at optimizing dose, regimen, schedule, and adjuvant, followed by monkey/SHIV and chimpanzee/HIV immunogenicity and challenge studies are now being planned.

6.2 Post-exposure prophylaxis of primary isolate HIV infection in chimpanzees

Following up from successful SCID-Hu postexposure prophylaxis described in the last annual report (see Annual Report, year 2, May 96-April97), studies were undertaken to evaluate the effect of B4 on infection of chimpanzees with primary isolate DH-12 of HIV-1. Preliminary studies (data not shown) demonstrated that B4 was effective at concentrations <1μg/ml in blocking HIV DH-12 infection of chimp PBL. Table 6 demonstrates the efficacy of both monoclonal B4, and sera from guinea pigs immunized with either peptide 2057c or 2240c to neutralize HIV DH-12 in an MT-2 neutralization assay.

MATERIALS AND METHODS

Animals. (4) chimpanzees, *P. troglodytes,* >5 years of age, were studies in an IUCAC approved protocol conducted at the Southwest Foundation of Biomedical Research, San Antonio, Texas, in collaboration with Dr. Kris Murthy.

Study Protocol. Treatment groups were assigned as follows: (1) Control; (1) pre-treated 1 hr. prior to HIV infection with 5mg/kg B4; (2) treated 1hr post HIV infection with 5mg/kg B4. Virus challenge with $100TCID_{50}$ of HIV-1 DH12 (Shibata et. al) intravenously, using a cell-free challenge. Blood samples will be collected prior to treatment, post treatment (prior to virus challenge), and 2 days post treatment, followed by weeks 1,2,3,4,6,8,10,12,14,16,18,20. Lymph node biopsy sample will be collected at weeks 4 and 20. Comprehensive virological and immunological studies will be done including, but not limited to: virus co-culture; PCR, ELISA, neutralization assays to monitor infection.

RESULTS:

Table 7 demonstrates that through 6 weeks of study, only the control chimpanzee shows evidence of HIV infection, whereas the chimps treated either one hour prior to virus challenge or one hour post virus challenge remain virus-free. Although we continue to monitor this study closely, based on these promising observations we have initiated discussions with the US Food and Drug Administration regarding the design of clinical trials to evaluate safety and pharmokinetics of B4 for use as a post-exposure prophylaxis for emergency exposure to HIV.

## 7.0 CONCLUSIONS

### A. Summary of major observations from year 3: May'97-April '98

• Peptide mimetics have been designed with the capacity to stimulate antibodies capable of neutralizing divergent primary isolates of HIV

• Monoclonal antibody B4 successfully prevented the establishment of primary isolate infection of HIV-DH12 in chimpanzees, even when the monoclonal antibody was added 1 hour post viral challenge.

These findings provide a key lead toward the development of immunogens capable of generating the appropriate humoral immune responses likely to be required for protective immunity against HIV, and for initiating combination studies aimed at complementing such humoral immune responses with cellular immunity capable of targeting and eliminating HIV infected cells.

### B. Future Directions

Despite a clear understanding of the correlates of protective immunity against HIV, it is generally viewed that vigorous humoral (neutralizing antibody versus clinical isolates) and cellular (T-helper and CTL) responses will be needed for protection (Burton and Moore, 1998), and that generation of such responses in both systemic and mucosal compartments would enhance the potential for success.

The capacity to induce functionally relevant neutralizing antibodies i.e. those which can neutralize across a broad spectrum of globally divergent isolates, has thus far been a major obstacle in the path towards an effective AIDS vaccine. There is evidence in some HIV/SIV animal models that neutralizing antibodies can protect against HIV infection (Burton et. al., 1997), but antibodies induced by first generation HIV vaccines have been ineffective at neutralizing primary isolates. Even the best of the anti-HIV monoclonal antibodies identified have only neutralized a narrow spectrum of clinical isolates, and usually at concentrations not likely to be achieved by vaccination (Trkola et. al., 1995).

In contrast, the identification of monoclonal B4 with its broad spectrum anti-HIV neutralizing profile has now led to the identification of prototype synthetic peptide immunogens capable of neutralizing HIV-1 clades A-F thus far tested. These observations provide the basis for now proposing a spectrum of vaccine development studies aimed at optimizing the immunogenicity of these antigens, and then combining these immunogens with those vaccine strategies which elicit optimal cellular immune responses e.g. DNA plasmid and viral vector strategies. Thus, the goals for year 4 of this grant are:

1. Studies aimed at optimizing dose, regimen, schedule, and adjuvant, followed by monkey/SHIV and chimpanzee/HIV immunogenicity studies for peptide immunogens, with the goal of stimulating high titered and durable humoral immunity.

2. Completion of the chimpanzee trials with the monoclonal B4, and production of B4 under cGMP for an IND filing for Phase 1 toxicity/pharmacokinetics studies.

3. Based on effective non-human primate studies for neutralizing antibody induction (Goal #1), to undertake combination studies of DNA immunogens + peptide boosts for maximizing CTL and humoral immunity, and for stimulation of systemic/mucosal immunity.

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## 9.0 **APPENDIX (List of Tables)**

Table 1: Immunogenicity of representative linear peptides targeting HIV receptor/co-receptors

| Peptide # | Target | Anti-peptide ELISA[1] | Neutralization titer[2] |
|---|---|---|---|
| 1403 | CD4 | 4.3 | <1:10 |
| 1405 | CD4 | 4.3 | <1:10 |
| 1999 | CCR1 | >5.0 | <1:10 |
| 2086 | CCR2 | 4.3 | <1:10 |
| 2079 | CCR3 | 4.3 | <1:10 |
| 2045 | CCR5 | >5.0 | <1:10 |

[1] $\text{Log}_{10}$ ELISA anti-peptide reciprocal titer

[2] MT-2 neutralization assay of clade B primary isolate HIV-1 VL 135; serum dilution at 50% inhibition.

Table 2: Immunogenicity of representative cyclized or cross-linked peptides targeting HIV receptor/co-receptors

| Peptide[1] | Target | Anti-peptide ELISA[2] | Neutralization titer[3] |
|---|---|---|---|
| 1585c | CD4 | 4.3 | <1:10 |
| 1821x | CD4 | >5.0 | <1:10 |
| 2006x | CCR1 | >5.0 | <1:10 |
| 2091x | CCR2 | 3.5 | <1:10 |
| 2084x | CCR3 | 4.3 | <1:10 |
| 2049x | CCR5 | 4.3 | <1:10 |
| 1996x | LESTR | >5.0 | <1:10 |

[1] c= peptides cyclized through cysteines added on amino and carboxyl termini of peptide.
x= cross-linkage of two peptide chains through an interdisulfide bond

[2] $\text{Log}_{10}$ ELISA anti-peptide reciprocal titer

[3] MT-2 neutralization assay of clade B primary isolate HIV-1 VL 135; serum dilution at 50% inhibition.

Table 3: Neutralization of HIV primary isolate by peptide mimetics to HIV receptor/ co-receptors

| Peptide # | Anti-peptide ELISA[1] | Neutralization titer[2] |
|---|---|---|
| 1619c | >5.0 | <1:10 |
| 2057c | 4.5 | 1:165 |
| 2189c | >5.0 | 1: 23 |
| 2190c | >5.0 | 1: 15 |
| 2240c | 4.0 | 1:283 |

[1] $\text{Log}_{10}$ ELISA anti-peptide reciprocal titer

[2] MT-2 neutralization assay of clade B primary isolate HIV-1 VL 135; serum dilution at 50% inhibition.

Table 4: Cross-clade neutralization of HIV primary isolates by peptide mimetics to HIV receptor/co-receptors

| | 90% Neutralization titers | | | | |
|---|---|---|---|---|---|
| Sera | Clade A | Clade B | Clade C | Clade D | Clade E |
| p2057c | 1:20 | 1:157 | 1:184 | 1:20 | 1:187 |
| p2240c | 1:76 | 1:324 | NT | 1:20 | 1:102 |
| Mab B4 (μg/ml) | 6.76 | 1.54 | 2.82 | 25.6 | 3.32 |

Peptides p2057c and 2240c were immunized into Duncan Hartley guinea pigs and sera collected as described in Materials and Methods. Neutralization assays were conducted in MT-2 microplaque assay as described in Materials and Methods, with a 90% endpoint.

Table 5: Neutralization of SHIV primary isolates by sera from guinea pigs immunized with peptide mimetics to HIV receptor/co-receptors.

| | 50% Neutralization Titers | | |
|---|---|---|---|
| Sera | SIVmac 251 | SHIV89.6 | SHIV89.6P |
| p2057c | 1:543 | 1:125 | 1:65 |
| pre-bleed | NT | <1:20 | <1:20 |
| B4 | 1:3553 | 1:9234 | 1:7929 |

Sera was obtained from guinea pigs immunized with p2057c at week 8 as described in Materials and Methods. Neutralization of laboratory isolate SIVmac 251 is shown for comparison.

Table 6: Neutralization of HIV-1 DH12 by monoclonal antibody B4 or sera from peptide-immunized guinea pigs

| Sera | 90% Neutralization vs. HIV-1 DH-12 |
|---|---|
| p2057c | 1:20 |
| p2240c | 1:36 |
| B4 | 2.1μg/ml |

Sera was obtained from guinea pigs as described in Materials and Methods; Neutralization assays were conducted in MT-2 cells as described in Materials and Methods.

Table 7: Post-exposure prophylaxis of HIV-1 primary isolate infection of chimpanzees by monoclonal B4.

| Chimp #[1] | Virus Isolation (PBL)[2] | Virus Isolation (Lymph Node)[3] |
|---|---|---|
| 084 | - | - |
| 259 | + | + |
| 356 | - | - |
| 357 | - | - |

[1] Chimp # 259: Control
# 084: Pre-treated 1hr prior to viral challenge with 5mg/kg B4
# 356, 357: Treated 1hr post viral challenge with 5mg/kg B4
Viral challenge: $100TCID_{50}$ of HIV-1 DH12 i.v.

[2] Virus isolation was done by co-cultivation of PBL with a Coulter p24 ELISA endpoint. Data represents week 6 post viral challenge; data for weeks 1,2,4 post viral challenge showed a similar profile.

[3] Lymph node biopsy was done at 4 weeks post viral challenge, and will be done again at 20 weeks. PCR and serology data is pending.